

April 3, 2002

PRELIMINARY AMENDMENT
Patent Application
Docket No. GJE-89

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Daniel Henry Densham
Docket No. : GJE-89
For : DNA Sequencing Method

Box PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

It is respectfully requested that the above-identified patent application be amended as follows:

In the Specification

After page 15: Please insert as new page 16 the attached Abstract of the Disclosure.

In the Claims

Please cancel claims 1-21, without prejudice.

Please add the following new claims 22-45:

22. A method for determining the sequence of a polynucleotide, comprising the steps of:
 - i. reacting a target polynucleotide with an enzyme that is capable of interacting with and processing along the polynucleotide, under conditions sufficient to induce enzyme activity; and
 - ii. detecting conformational changes in the enzyme as the enzyme processes along the polynucleotide.

23. The method according to claim 22, wherein the enzyme is a polymerase enzyme.
24. The method according to claim 22, wherein the enzyme is a helicase enzyme or a primase enzyme.
25. The method according to claim 22, wherein the enzyme is immobilised on a solid support.
26. The method according to claim 25, comprising a plurality of enzymes immobilised on the solid support.
27. The method according to claim 22, wherein the enzyme comprises a first bound detectable label, the characteristics of which alter as the enzyme undergoes a conformational change.
28. The method according to claim 27, wherein the enzyme comprises a second bound detectable label capable of interacting with the first label, wherein the degree of interaction is dependent on a conformational change in the enzyme.
29. The method according to claim 27, wherein a second detectable label is bound to a nucleotide brought into contact with the enzyme.
30. The method according to claim 28, wherein the first label is an energy acceptor and the second label is an energy donor, or wherein the first label is an energy donor and the second label is an energy acceptor, and wherein step (ii) is carried out by measuring energy transfer between the two labels.

31. The method according to claim 29, wherein the first label is an energy acceptor and the second label is an energy donor, or wherein the first label is an energy donor and the second label is an energy acceptor, and wherein step (ii) is carried out by measuring energy transfer between the two labels.

32. The method according to claim 22, wherein step (ii) is carried out using confocal microscopy.

33. The method according to claim 32, wherein step (ii) is carried out by fluorescence imaging.

34. The method according to claim 27, wherein step (ii) is carried out by measuring a polarisation effect consequent on the altered characteristics of the first label.

35. The method according to claim 34, wherein step (ii) is carried out by fluorescence polarisation anisotropy.

36. A method for determining the sequence of a polynucleotide, comprising detecting via fluorescence resonance energy transfer a conformational change in an enzyme that interacts with and processes along a target polynucleotide, thereby permitting determining the sequence of the polynucleotide.

37. The method according to claim 36, wherein the enzyme is a polymerase enzyme.

38. The method according to claim 36, wherein the enzyme is immobilised on a solid support.

39. The method according to claim 37, wherein the enzyme is immobilised on a solid support.

40. A method for determining the sequence of a polynucleotide, comprising detecting a detectably-labelled enzyme that is capable of interacting with and processing along a target polynucleotide, wherein the label alters its detectable characteristics as the enzyme processes along the polynucleotide, thereby permitting determining the sequence of the polynucleotide.

41. A solid support comprising at least one immobilised enzyme capable of interacting with and processing along a target polynucleotide, the enzyme being labelled with one or more detectable labels.

42. The solid support according to claim 41, wherein the enzyme is a polymerase.

43. The solid support according to claim 41, wherein the label is a fluorophore.

44. The solid support according to claim 42, wherein the label is a fluorophore.

45. A system for determining a sequence of a polynucleotide, comprising a solid support according to claim 41, and an apparatus for detecting the label.

Remarks

Support for the new claims presented herein can be found throughout the subject specification and claims 1-21 as filed.

The Commissioner is hereby authorized to charge any fees under 37 CFR §§1.16 or 1.17 as required by this paper to Deposit Account No. 19-0065.

Respectfully submitted,



Doran R. Pace
Patent Attorney
Registration No. 38,261
Phone No.: 352-375-8100
Fax No.: 352-372-5800
Address: 2421 N.W. 41st Street, Suite A-1
Gainesville, FL 32606-6669

DRP/sl

Attachment: Abstract of the Disclosure

Abstract of the Disclosure

The present invention pertains to a method for determining the sequence of a polynucleotide, the method relying on the detection of a conformational change in an enzyme that interacts with and processes along the polynucleotide. The detection of a 5 conformational change may be carried out by measuring changes in a fluorophore bound to the enzyme.